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for

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on

USE OF FOOD COLOURS TO DYE ENZYME SOLUTIONS

by

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Use of food colours to dye enzyme solutions

The present invention relates to the use of food colours to dye enzyme solutions, to enzyme solutions provided with food colours as well as their use.

DE 197 37 173 A1 relates to a microdispensing system for the dispensing of very small amounts of liquid. The system is used for example in molecular biology to carry out enzymatic reactions. The very small amounts of liquid (e.g. enzyme solutions) are fed in measured quantities into a reaction vessel using a free-jet dosing apparatus e.g. electrostatically, piezoelectrically or thermomechanically, the nozzle of the free-jet dosing apparatus having to be positioned exactly centrally over the reaction vessel, in order to guarantee that the dispensed liquid is actually introduced into the reaction mixture and does not remain completely or partly suspended on the wall of the reaction vessel, without coming into contact with the solution.

The object of the present invention is thus to create a possibility for the person skilled in the art to test the dispensing of the amounts of liquid using the

aforementioned microdispensing system, i.e. in order to be able to judge whether the dispensed sample volumes have also actually been completely added to the reaction mixture.

The object is achieved according to the invention by an enzyme solution which contains one or more food colours, preferably one or more food colours according to Annex I for EC directive 94/36 of 30.06.1994 (in the version published on 10.09.1994 in the Official Journal No. L 237 (p. 0013-0029) of the European Communities), which is incorporated herein by reference. Among the particularly preferably used food colours are azo dyes and triarylmethane dyes which are soluble in water and distinguish themselves among food colours due to their depth of colour, i.e. the very high molar extinction coefficient. Only small amounts of dye are necessary, in order to achieve a sufficient colouring (cf. e.g. Ullmann - Encyklopädie der technischen Chemie, 4th Edition, Verlag Chemie 1974, Volume 8, pp. 260 ff).

According to a particularly preferred version of the invention, the food colours are selected from the group consisting of Ponceau 4R (E124), Patent Blue (E131) and tartrazin (E102). Practically any shades can be mixed from these three basic colours.

There are no limitations whatsoever with regard to the enzyme. The enzyme solution contains one or more enzymes, such as e.g. enzymes from the group consisting of restriction enzymes and polymerases.

The enzyme solutions according to the invention are characterized in particular in that they show no loss of

activity even during prolonged storage periods (or only a slight loss of activity, mainly under 20%).

With regard to the amount of colour used, there are no limitations whatsoever according to the invention. For use in conjunction with the microdispensing system of DE 197 37 173 A1, a minimum quantity has proved to be advantageous which allows a colouring of a drop to be just recognisable visually against a white background. It is not necessarily required that a shade can also still be recognised after mixing the dyed enzyme solution with the reaction mixture.

The invention further relates to the use of the above-named enzyme solutions in a microdispensing system according to DE 197 37 173 A1, which is incorporated herein by reference. The dying of the enzyme solution produces the following advantages as regards handling: The very small drops (up to 10nl) dispensed using this system can be recognised only with difficulty when uncoloured. The dying of the enzyme solution facilitates the effortless control of the results, namely a) whether the drop was dispensed and b) whether it reached the correct position.

The subject-matter of the present invention is thus furthermore the use of the above-named food colours for the preparation of enzyme solutions, preferably for use in a microdispensing system according to DE 197 37 173 A1. The enzyme solutions can contain one or more enzymes, such as e.g. from the group consisting of restriction enzymes and polymerases.

The invention is explained in more detail in the following examples.

Examples:

1) Materials and methods:

Restriction enzymes (Roche Basel, Switzerland) and dilution buffers:

Bam H1, 10 units/µl (No. 567 604, dilution buffer = P2)
Cla 1, 10 units/µl (No. 404 217, dilution buffer = P1)
Dra 1, 10 units/µl (No. 779 695, dilution buffer = P1)
Eco R1, 10 units/µl (No. 1 175 084, dilution buffer = P3)

Colours:

All colours from Synopharm, 22887 Barsbüttel, Germany.

Ponceau 4R, No. 145 130, batch: 94030770
Patent blue, No. 700 141, batch: 95060280
Tartrazine, No. 145 160, batch: 96040020

Preparation of the dyed enzyme solutions

The three colours red, blue and yellow, and the mixture blue and yellow for the preparation of green, were added to the dilution buffers P1, P2 or P3 to be used according

to the instructions of the manufacturer Roche to 0.5 % (w/v) colour content.

1 part of corresponding, dyed dilution buffer was added to 9 parts of an enzyme solution. The colour of the dilution buffer corresponded to the code colours for the reaction buffers allocated to the enzymes (blue for Bam H1, red for Cla 1, green for Dra 1, red for Eco R1).

Yellow is thus tested in the combination with blue.

Dyed enzyme solutions were thus prepared which contained 500 ppm colour.

Mixtures of 9 parts of the enzyme solutions used with 1 part of the corresponding undyed buffer were used as reference.

Measurement of the enzyme activity:

The enzyme activity was measured by digestion of λ-DNA in the form of a time series.

The rate of reaction of the dyed enzyme solution can be compared with the undyed reference from the gelectrophoretic image of the reaction stopped at 8 to 10 successive time points. The rate of reaction is at a first approximation inversely proportional to the time which is required for the digestion of the substrate.

The Roche buffers, modified by an addition of 0.02 % Tween 20 and 2% of the corresponding dilution buffers, were used as reaction buffers.

2) Results:

Figures 1 to 4 show the rates of the digestion of λ-DNA for the 4 enzymes after 1 week's storage at -21°C, Figures 5 to 8 after almost a half-year's storage at -21°C.

A change in the activity of the enzymes caused by the presence of the colours used cannot be recognised, at both points in time the rate of digestion by dyed and undyed enzyme solution is the same.